FEASIBILITY STUDY ON LIQUID GLUCOSE PREPARATION USING CASSAVA

by

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Declaration

The work described in this thesis was carried out by me at the Food Research Unit, Department of Agriculture, Gannoruwa, Peradeniya under the supervision of Mr. S. Ekanayake and Mr. J. Wansapala. A report on this has not been submitted to any other university for another degree.

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AFFECTIONATE DEDICATION TO
MY PARENTS AND TEACHERS

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Abstract

Liquid glucose is an essential ingredient used in confectionery and other food preparation industries mainly to prevent crystallization of sugar and consequence hardening. Over 3, 000 Mt of liquid glucose are imported to Sri Lanka annually and it is cost about Rest. 100,000,000/=. The purpose of this study is to see the feasibility of the production of liquid glucose for cottage and medium scale industries by acid hydrolysis and reduce the importation cost.

In this study starch of cassava, which has bulk production in Sri Lanka is used as the raw material. Hydrolysis of starch to glucose is achieved using hydrochloric acid. (Acid hydrolysis) After neutralization with sodium carbonate, the hydrolysis is decolorized, filtered and concentrated to obtain a product containing 80-85% total solids.

The starch samples were acidified with 2M, 4M and 6M of hydrochloric acid. The samples treated with one acid concentration were autoclaved at three different pressure levels $68.94x10^3$ Nm^{-2}, $103.41x10^3$ Nm^{-2} and $137.88x10^3$ Nm^{-2} and the hydrolyzing time was measured at 120 ^0C. The TSS, Dextrose Equivalent, color, moisture content and ash content of the prepared liquid glucose sample were analyzed.

A sensory evaluation was carried out for two jujubes samples, which were prepared using market liquid glucose and prepared liquid glucose and compared the organoleptic properties of both products.

From the findings of this study, it can be concluded that the best combination of acid concentration and pressure level for the optimum hydrolysis of cassava starch at 120 ^0C is 6M and $137.88x10^3$ Nm^{-2} respectively. The prepared liquid glucose is grayish yellow in colour, Dextrose Equivalent value is 37.9 and the TSS is 82%. Also the moisture content and the total ash content of the prepared liquid glucose sample are 16.52% and 2.73% respectively.

The results of the sensory evaluation reveal that there is no significant difference of organoleptic properties i.e. colour, smell, texture, taste, appearance and acceptability between the two jujubes samples. It implies that there is no significant difference of functional properties between liquid glucose available in the market and prepared liquid glucose using cassava starch.

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Abbreviations

CG – Cyanogenic Glucosides

DE – Dextrose Equivalent

e.g. – Example

HCN – Hydrogen Cyanide

i.e. – That is

LA – Lactic Acid

LAI – Leaf Area Index

s.g. – specific gravity

Chapter 1

Introduction

1.1 Introduction

Liquid glucose, which is also known as glucose syrup, starch syrup, and commercial glucose is highly viscous, slightly sweet and colourless substance. It is a concentrated aqueous solution containing glucose, maltose, dextrin and other oligosaccharides. (Bean and Sester , 1992)

The production of liquid glucose was first come into existence in the field of carbohydrate sweetening soon after the Second World War. (Radley , 1976) The uniqueness of liquid glucose is its high viscosity, sticky nature and ability to control sweetening in confectionery industry. At present liquid glucose is extensively used as sweetening agent in confectionery, bakery and other food preparation. Also it is used to prevent crystallization of the sucrose present and produce a clear non-crystalline product in boiled sweets, candies and other types of confectioneries. In addition it contributes to body, mouth appeal and shelf life of the sweets.

Annually about 3,000 – 4,000 Mt of liquid glucose are imported to Sri Lanka from other countries such as France, Thailand, Pakistan, India, Germany, Singapore and Italy. (External Trade Statistics , 1995-2002). Therefore manufacturers in confectionery and other food production industries have to spend lot of money for the importation of liquid glucose.

Starch is the starting raw material for making liquid glucose. Starch is commonly produced from Corn Wheat and tubers (eg :- sweet potato, cassava, potato) and isolated from the plant source as an aqueous suspension of glucose and the conversion of starch to glucose can be carried out by both acid hydrolysis and enzyme hydrolysis.

Cassava (*Mannihot esculenta*.Crants.) also known as "yuca", "tapioca", "manioc", or "mandioca" is a high carbohydrate tropical root crop cultivated for its starchy tuberous roots. Cassava cultivation in Sri Lanka is primarily concentrated in the wet

and intermediate zones. (Jayawardana , 1986) According to the statistics, extension of cassava is around 30, 000 Ha in Sri Lanka and the annual production is 250, 000 Mt in last ten years. (Department of census statistics , 1997-2002)

In Sri Lanka commonly cassava is consumed by cooking and boiling after removing the rind and less or no industrial utilization. Therefore it is more economical to produce liquid glucose using cassava as a source of inexpensive starch.

1.2 Objectives

1) Optimization of the acid concentration and pressure level required for the complete hydrolysis of starch to produce liquid glucose.
2) Production of liquid glucose for cottage and medium scale confectionery industries.

1.2 Specific objectives

1) Utilization of bulk production of cassava in industrial level and reduce the postharvest losses
2) Value addition to the crop.
3) Reduce the high importation cost for liquid glucose.

Chapter 2

Literature Review

2.1 Introduction to cassava

2.1.1 Origin and distribution

The cassava plant originated in North East Brazil and in Central America. (Onwueme , 1978) Today the plant has spread to various parts of the world and cultivated in all tropical regions in the world. To the Indian sub-continent, the crop was introduced by the Portuguese during the early 18th century. Sri Lanka, officially imported cassava cuttings from Mauritius in 1786 and India made importations directly from South America in 1974 and from West Indies in 1840. (Nayar , 1986)

In Sri Lanka cassava is cultivated mainly in the wet and intermediate zones during both Yala and Maha seasons as shifting cultivation or in home garden. In dry zone it is cultivated mainly in Maha season. The largest area of cassava cultivated is Kurunegala district and mostly intercropped with coconut. Also in Gampaha district it is intercropped with pineapple. (Jayawardena , 1986)

Table 2.1 Annual extension, production and average yield of cassava in Sri Lanka

Year	Extension (Ha)	Production (Mt)	Average Yield (Mt/Ha)
1997	28,855	249,779	8.66
1998	30,064	257,153	8.55
1999	29,432	251,513	8.55
2000	29,543	249,110	8.43
2001	27,301	233,579	8.56
2002	26,380	224,986	8.53

Source: (Agricultural statistical data, Department of Census Statistics, 1997-2002)

2.1.2 Taxonomy

Cassava is a dicotyledonous plant belonging to the

Tribe – Manihotae

Family – Euphorbiaceae

Sub-family – Crotonoideae

Genus – Manihot

Manihot genus is reported to have about 100 species. Among them *Manihot esculenta* Crants. exists only as a cultivated species. (Nayar , 1986)

2.1.3 Cassava cultivars

Numerous cassava cultivars exist in the world and the cultivars have been distinguished on the basis of morphology (e.g. :- leaf shape and size, plant height, petiole colour), tuber shape, earliness of maturity, yield and the content of cyanogenic glucoside of the root. (Onwueme , 1978)

According to the cyanogenic glucoside content, cassava cultivars can be grouped into two groups.

- Bitter varieties :- In bitter varieties the cyanogenic glucoside is distributed throughout the tuber at a high level i.e. higher than 100 mg/kg. (Alves , 2002) The bitter cassava require 12 - 18 months to mature and will not deteriorate if left unharvested for several months.

- Sweet varieties :- In sweet varieties the cyanogenic glucoside is confined mainly to the peel at a low level i.e. less than 100 mg/kg. (Alves , 2002) Therefore the flesh is relatively free of glucosides. Sweet cassava has a short growing season and their tubers mature in 6-9 months and deteriorate rapidly if not harvested soon after maturity. Most of the varieties cultivated in Sri Lanka are sweet varieties.

2.2 Environmental conditions required for cassava cultivation

- **Temperature**

  Cassava is a crop of the low land tropics. It grows best in a warm moist climate where temperature ranges from 25 ^{0}C – 29 ^{0}C. It grows very poorly under cold climates and at temperatures below 10 ^{0}C growth of the plant is arrested. It cannot withstand frost at any time during its active growing period. (Onwueme , 1978)

- **Rainfall**

  Cassava grows best when the rainfall is 100 - 150 cm per year and well distributed. However the crop is well adapted to cultivation under drought conditions where the annual rainfall is as low as 50 cm. (Onwueme , 1978)

  When moisture availability is low, the cassava plant ceases growth and sheds some of its older leaves thereby reducing its total transpiring surfaces. When moisture is again amply available, the plant quickly resumes growth and produce new leaves. This behavior makes cassava a valuable crop in places where the rainfall is low and uncertain or both. The cassava plant is unable to tolerate drought conditions only during the first few weeks after planting.

- **Soil**

  The best soil for cassava cultivation is a light sandy loam soil of the medium fertility. Good drainage is also important. On clay or poorly drained soils, root growth is poor, so that the tuber-to-shoot ratio is considerably decreased. Gravelly or stony soils tend to hinder root penetration and saline soils are also unsuitable.

  Cassava can grow and yield reasonably well on soils of low fertility where production of most other crops would be uneconomical. Under conditions of very high fertility cassava tends to produce excessive vegetation at the expanse of tuber formation. (Onwueme , 1978)

➢ **Light**

Tuber formation in cassava is under photoperiodic control. Under short-day conditions tubering occurs readily. But when the day length is greater than 10 - 12 hours tubering is delayed and subsequently yield is lower. For this reason cassava is most productive between latitudes 15 ^{0}N and 15 ^{0}S. (Onwueme , 1978)

2.3 Morphology, growth and development of cassava tuber

Root formation in cassava takes place both from the nodes and the stem cutting used for propagation. Under ideal conditions the first roots emerge within 5 days after planting. After a few weeks, some roots exhibit secondary growth in the xylem. The number of roots that begins secondary thickening and tuber size is determined by the genotype, carbohydrate supply and hormonal factors. Root elongation slows down once the secondary thickening commences. Starch content also increases, with the enlargement of the tubers and reaches the peak between 7th to 10th months after planting. Thereafter the starch content decreases and fiber content increases. (Nayar , 1986)

A mature cassava tuber (excluding the tail) may ranges in length from 15 - 100 cm in diameter from 3 - 15 cm and in weight from 0.5 - 2.0 kg depending on variety and growing conditions.

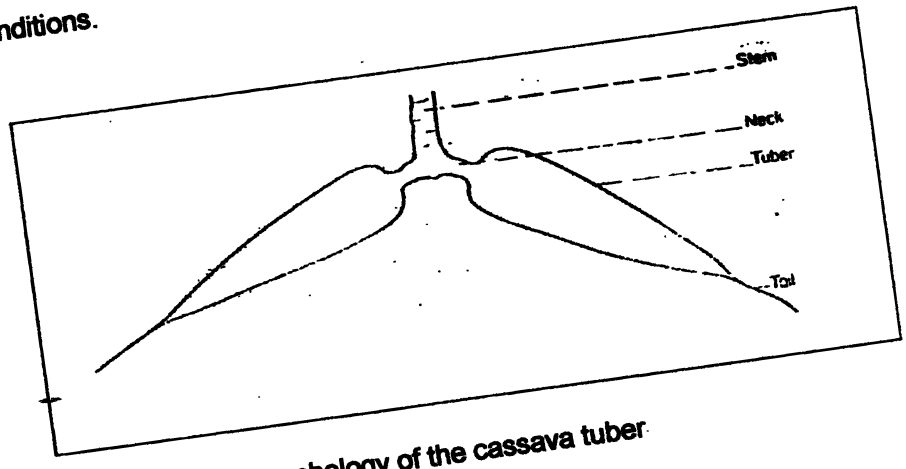


Figure 2.1 General morphology of the cassava tuber

Source: (Onwueme , 1978)

The cassava tuber is circular in cross section. It is generally fattest at the proximal end, and tapers towards the distal portion.

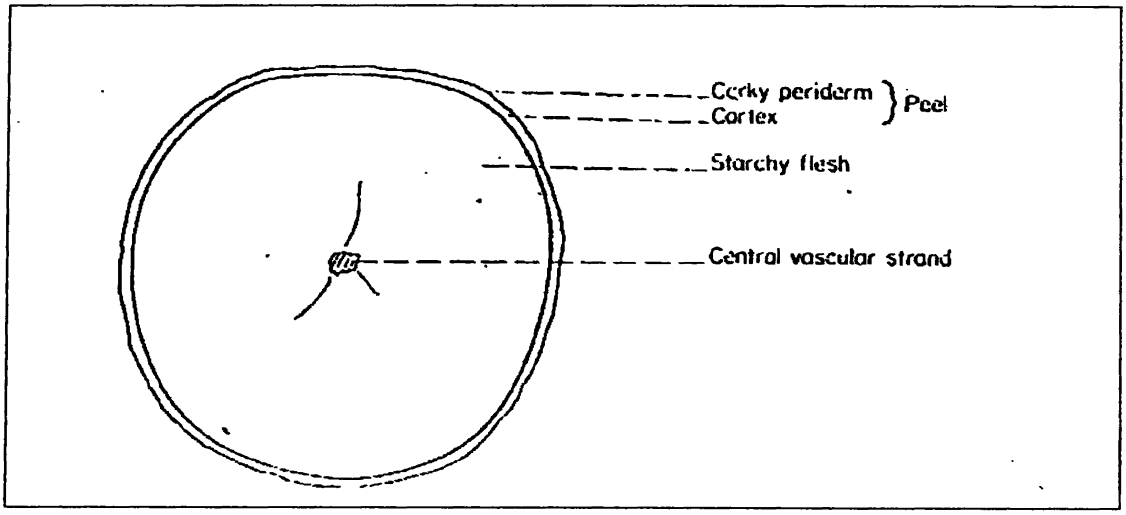


Figure 2.2 Transverse section of the cassava tuber
Source: (Onwueme , 1978)

In internal structure, the tuber is divided into three regions.

1) Periderm :- The outer most layer, and composed of dead cork cells, which effectively seal the surface of the tuber. The thickness of the periderm is only a few layers of cells. As growth progresses, the outer most portions of it are sloughed off and replaced by new cork formation from beneath.

2) Cortex :- Just beneath the periderm presence the cortex which is only 1 - 2 mm thick. It is usually white but sometimes may be pinked or brownish. The peel of the cassava tuber is composed of the cortex with the outer periderm layer adhering to it.

3) Starchy flesh :- The central portion of the tuber, which is constituting the bulk of the tuber. This consists mostly of parenchymatous cells containing large amounts of stored starch. Thin vascular bundles randomly branched out throughout the flesh and a large strand of vascular tissues run through the center of the flesh. Latex tubes (laticifers) occur in the flesh of the tubers. (Onwueme , 1978)

2.4 The composition of cassava tubers

The dry matter content and composition of cassava tubers are influenced by the environmental factors, the variety and the stage of harvest. In general the tubers contain 30 - 40% carbohydrate, 1 - 2% crude protein, 0.2 - 0.6% other extractives and 55 - 60% moisture.

The carbohydrate fraction is primarily composed of 90% starch. Cassava starch has 20% amylose and 80% amylopectin. The protein accounts for only 40 - 50% of the total N_2 in the tuber and the biological value of the protein is 48%. Among the minerals, cassava contains phosphorus and calcium in sufficient amounts, but is deficient in others. The tuber also contains significant quantities of vitamin C, thiamine, riboflavin and niacin. The metabolizable energy of dry cassava is 3500 - 4000 kCal/g and considered as a good source of energy. (Nayar , 1986)

Table 2.2 Approximate composition of cassava tuber

Principles	Composition
Major constituents:	
Moisture (%)	59.4
Carbohydrates (%)	38.1
Protein (%)	0.7
Fat (%)	0.2
Crude fiber (%)	0.6
Ash (%)	1.0
Minor constituents:	
Calcium (ppm)	500
Phosphorus (ppm)	400
Iron (ppm)	9
Thiamine (ppm)	0.50
Vitamin C	250

Source : (Nayar , 1986)

2.5 Cyanogenasis of cassava

Cassava tissues contain natural nitriles (-CN) compounds called cyanogenic glucosides (CG) which limits the food and feeding value. All organs except seeds contain CG. The most abundant CG is linamarin (85%) with lesser amount of lotaustralin. (Alves , 2002)

Total CG concentration in cassava depends on

- Cultivar
- Soil – Plant growing on soils low in P and high in N_2 have high HCN concentration.
- Region – Cassava grown in wet region has a high HCN content than that grown in dried region.
- Age – As the plants get older, the HCN content increases, attains a peak and then begins to decline. But the exact age of the peak may vary with cultivars.

These CGs are synthesized in the leaf and transported to the roots and converted to HCN, which is highly poisonous to human and animal, when they contact with enzyme linamarase found in cassava tissues.

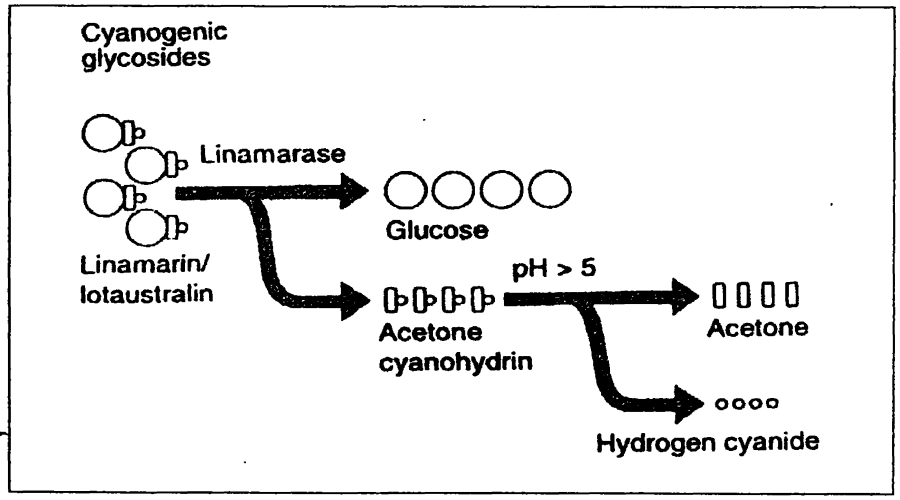


Figure 2.3 Cyanogenasis in cassava
Source: (Ekanayake *et al* , 1998)

The leaves especially younger leaves contain more cyanogenic glucosides than the other parts. In the tuber the peel contains significantly higher amounts of glucosides than the flesh. Also the activity of endogenous enzyme linamarase is higher in the peel. (Nayar , 1986)

Both glucosides are highly soluble in water, and tend to decomposer when heated to temperature above 150 ^{0}C. Also cooking in water for about 5 minutes removes more than 80 % of the cyanide. Sun drying of the tuber slices destroy about 15% of the total cyanide. As well as microbial detoxification and fermentation (Alves , 2002) can be achieved to reduce cyanide content to safe level.

2.6 Cassava utilization

In the past, the importance and potential of cassava were not adequately recognized. Now cassava is considered as an important food crop and there is greater interest in its commercial exploitation in animal feed and starch-based industries.

Uses of cassava vary greatly from region to region. Globally 60% of cassava production is used for human food, 27.5% for animal feed and the rest for starch and starch based industry. In Africa nearly all the cassava is used for human consumption. In Asia about 50% of total cassava production is for direct human consumption and the reminder is converted to dry chips, pellets or starch either for export or for used in local industries. (Nayar , 1986)

2.6.1 Utilization of fresh cassava tubers

It is common practice to eat raw cassava after removing skin and rind or cook by boiling or baking. There are two major factors affect to utilization of fresh cassava. They are –

- High amount of HCN content in unprocessed tuber
- Fresh cassava tubers cannot be stored for more than a few days after harvesting (Onwueme , 1978)

Mostly sweet cassava cultivars are grown for this purpose and eat raw cassava cultivars with high content of cyanogens must be cooked before consumption. Boiling cassava tubers in water destroy the enzyme linamarase and eliminates the HCN.

2.6.2 Culinary uses of cassava

Gari – The most popular form in which cassava is consumed in West Africa. It is a dry fermented and gelatinized coarse meal. Good quality gari is usually cream yellow in colour with uniform sized grain.

Farina – The most common meal in Brazil and some other parts of South America. It is very much similar to gari and consumed as combination with several other foods.

Cassareep – The juice pressed out from tubers during the preparation of farina is concentrated and added spices to make the sauce known as cassareep.

Attieke – A fermented, pre gelatinized meal, which is most commonly used in Cameroon and eaten with milk, meat or vegetables.

Fufu – It is a sticky dough eaten with soups made out of fish or vegetables and commonly used in West Africa and Ghana.

Cassava bread – A white, flat, circular, light textured bread baked from moist cassava pulp.

Macaroni – This is prepared by blending cassava flour, groundnut flour and wheat in the ratio 60:12:15. Also enriched macaroni containing proteins and fortified with vitamins and minerals has been developed for feeding children and vulnerable groups.

Manicuera – This is a boiled slightly sweet cassava drink available in the northwest Amazon region. (Balagopalan , 2002)

2.6.3 Cassava as an animal feed

Various part of the cassava plant including tubers, stems and leaves are used for feeding animals such as pigs, cattle, sheep and poultry. Cassava is an attractive ingredient in animal diet due to the high energy value and the low protein content is a disadvantage. This can be overcome by upgrading the feed with protein additives such as Soya.

2.6.4 Industrial uses of cassava starch

2.6.4.1 Adhesive

Adhesives are made by gelatinizing cassava starch by heat treatment. There are two types.

- Gums without additives – The liquid starch pastes are made by cooking starch with water and preservatives are added later. These are useful in bill pasting, bag making and in tobacco products
- Gums prepared using different chemicals – Various chemicals are added during the preparation of gums. These include inorganic salts like calcium and magnesium chlorides, borax, urea, glycerol and carboxylmethyl cellulose and useful in various applications such as lamination of papers, wall paper printing, water resistant formulations, pasting labels and other stationary applications. (Balagopalan , 2002)

2.6.4.2 Sago

Originally sago was derived from the palm, *Metroxylon* sp. found in Malaysia and Thailand. As well as sago can be manufactured using cassava starch and it is marketed as small globules or pearls. Sago is mainly used as infant and invalid food, and in preparation of puddings.

2.6.4.3 Liquid glucose

Starch is a polymer of glucose and the hydrolysis of starch to glucose can be carried out by acid hydrolysis and enzyme hydrolysis. The syrup can be used for various confectionary purposes, and after further purification is used for pharmaceutical purposes and for energy foods.

2.6.4.4 Fructose syrup

Fructose is 1.7 times sweeter than sucrose and four times sweeter than glucose. The conversion of glucose to fructose can be achieved by alkali or by the enzyme glucoisomarase.

2.6.4.5 Maltose

Maltose is a disaccharide and can be obtained commercially from starch by enzyme treatment. There are three types of maltose syrups, high maltose syrups, extremely high maltose syrups and high conversion syrups.

2.6.4.6 Maltodextrin

Maltodextrins are partially hydrolyzed starch with dextrose equivalent less than 20. They are used as food ingredients and manufactured by the action of α-amylase on starch. (Balagopalan , 2002)

2.6.5 Fermented commodity of cassava starch

2.6.5.1 Cassava alcohol: -

As cassava starch having lower swelling and gelatinization temperature, can be easily saccharified to simple sugars. Saccharified starch is inoculated with actively growing yeast (*Saccharomyces cerevisiae*) and allowed for fermentation.
(eg:- sorbitol , mannitol , moltol)

2.6.5.2 Citric acid: -

In the production of citric acid, cassava starch after gelatinization and liquefaction is subjected to fermentation using certain strains of *Aspergillus niger.*

2.6.5.3 Lactic acid (LA): -

Cassava starch can be utilized for the production of LA. The starch has to be saccharified into sugar before fermentation. The bacteria *Lactobacillus plantarum, L.bichmaina, L.masenterioides* and *L.delbruiki* can be used for fermenting saccharified starch to produce LA. (Balagopalan , 2002)

2.7 Starch

Starch is a plant polysaccharide found in roots, tubers and the endosperm of seeds as a chief carbohydrate reserve. It occurs in the form of granules which are usually an irregular rounded shape ranging in size from 2-100 μm. Both the shape and size of the granules are characteristic of the species of plants.(Coultale , 1996) Starch granules develop in cells called plastids during growth and maturation of the plant.

Pure isolated starch granules appear as a white, glistening, odorless and tasteless powder. Chemically starch consists of two glucose polysaccharides amylose and amylopectin. Both polysaccharides are built up entirely from D-glucose units. (more precisely D- glucopyranose). They occur together in granules, but amylose may be separated from starch solution since it is much less soluble in organic solvents such as butanol. (Coultale , 1996)

Amylopectin is the major polymer in most starches and represents 72-85% of the total starches. (Bean and Sester , 1992) In most starches 20-25% is amylose but there are exceptions. (e.g.: - Pea starch contains around 60% amylose)

2.7.1 Nature of amylose

Amylose is essentially a linear polysaccharide and consists of long chains of α-D-glucopyranosyl residues linked between their 1 and 4 positions, which are called as glycosidic bonds. (See figure 2.4)

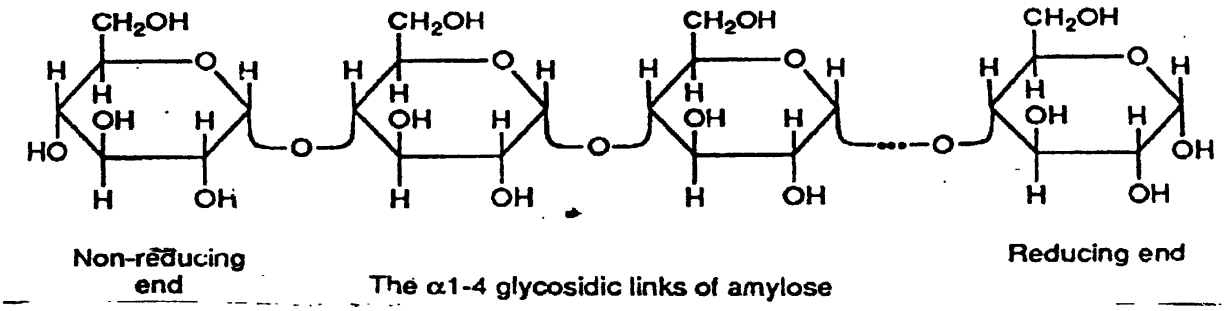


Figure 2.4 Molecular structure of amylose
Source : (Coultale , 1996)

Generally amylose composed of thousands of glucose units, so that molecular weights for amylose range from a few thousands to 150,000. (Zapsalis and Beck , 1986)

Amylose responsible for the gelation of cooked, cooled starch paste and is form a three dimensional network when molecules associate upon cooling. Examples of the amylose content of various starch sources include, cereal grains 26-28%, roots and tubers 17-23% and waxy varieties of starch 0%. (Vaclavic , 1998)

2.7.2 Nature of amylopectin

Amylopectin is a highly branched polysaccharide. It is a much larger molecule, having about 10^6 glucose units per molecule. The glucose units of amylopectin are joined by α 1-4 glycosidic links with α 1-6 links which creating branching points at every 15 - 30 glucose units of the chain. (Figure 2.5)

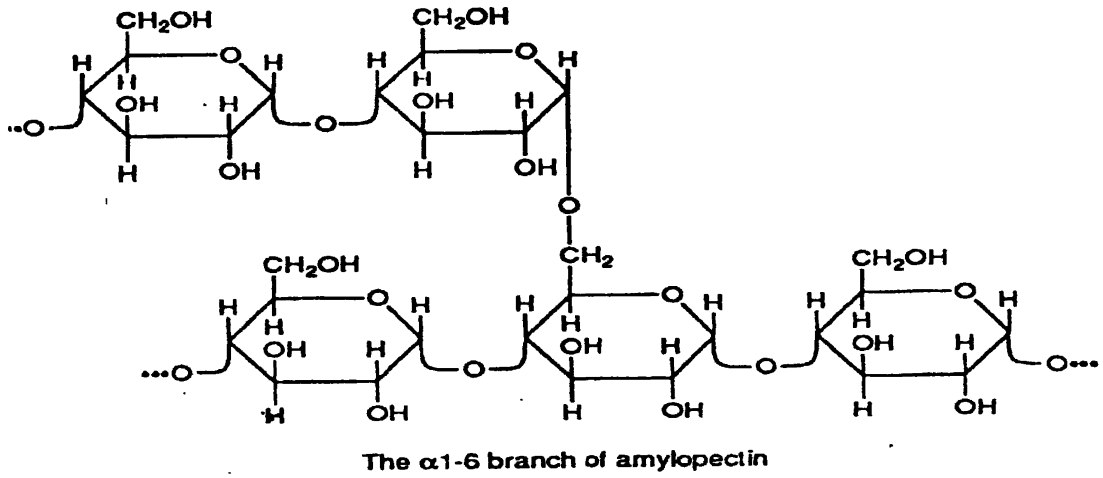


Figure 2.5 Molecular structure of amylopectin
Source: (Coultale , 1996)

The molecular weights for amylopectin vary widely and often exceed 500,000. (Zapsalis and Beck , 1986) Starch with high percentage of amylopectin will thicken the mixture but will not form gel because they do not associate like in amylose. (Voclavic , 1998)

Many hypotheses have been put forward to describe the arrangement of the branching of chains of amylopectin and the tree like arrangement first purposed by Mayer and Bernfled in 1940 was accepted. (Coultale , 1996) However the availability of the enzyme pullulanase, which is specific for the ∝1-6 linkage and gel filtration methods for the determination of the chain lengths of the resulting fractions have given the new insights. Following figure shows one possible arrangement of the chains, which is gaining wide acceptance. (Coultale , 1996)

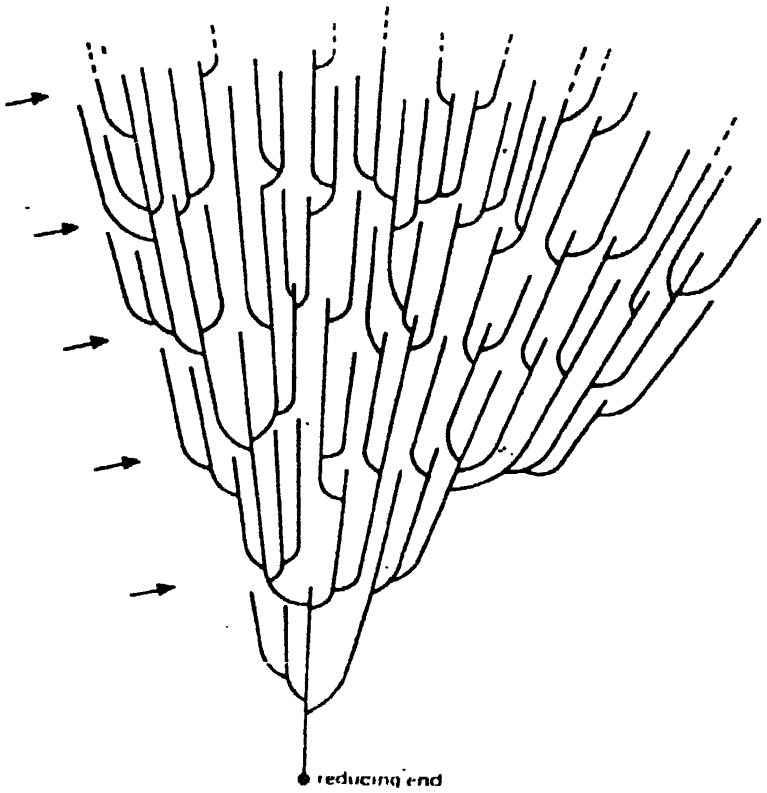


Figure 2.6 Arrangement of the branched amylopectin chains
Source: (Coultale 1996)

2.8 Physico-chemical properties of starch

In granule form, starch is quasicrystaline, water insoluble and dense. It is hydrated to only a small degree, so that a large amount of carbohydrate is stored in a small volume. The starch of each botanical species has characteristic features, which are differ from another species.

The molecular organization within the granule as well as the chemical characteristics of amylose and amylopectin components controls the important physical properties of starch.

2.8.1 Granule size and shape

It can be observe a wide difference of size and shape in the starch granules. Most common starches have a continuous size distribution with one predominant shape. Diameter of granules obtained from different species varies widely as given in the table 2.3. As well as geometric shape of the different tuber starches vary widely from polygons, ellipsoids, perfect spheres and disks.

Table 2.3 Granule size and shape of different starches

Species	Granule size (μm)	Granule shape
Cassava	5-35	round , flat one end
Colocasia	1-10	mostly round
Potato	15-110	oblong oyster shape
Maize	1-10	round polygonal
Rice	2-15	round polygonal
Corn	5-25	round polygonal
Wheat	2-10	round
	20-35	elipsoid

Source: (McNicol *et al.* , 1972 and Moorthy , 1995)

2.8.2 Colour and appearance

Among the tuber starches, the cassava starch is pure white in colour due to low contents of protein and phenolic compounds. Other tuber crop starches are not so white due to contamination with other ingredients. (Moorthy , 1995)

2.8.3 Starch content

Among the tuber crops cassava has the highest starch content. The starch content varies to a large extent in different crops as given in the table.

Table 2.4 Starch content of different species

Species	Starch (%)
Cassava	25-40
Sweet potato	20-30
Colocasia	12-22
Dioscorea alata	18-30

Source: (Moorthy , 1995)

2.8.4 Clarity and paste stability

This property of starch paste depends on the association forces in the starch. Generally starches having higher amylose content possess poor clarity and paste stability owing to the more close network of association forces.

A high degree of clarity is preferred for fruit products and an opaque, creamy appearance is desired for cream sauce. Clarity is influenced by the retrogradation tendency of the starch. (Bean and Sester , 1992)

2.8.5 Viscosity

A starch suspension in water starts swelling by imbibing water and increasing in size. The resulting solution is composed of a network of swollen granules, broken-down fragments of starch and leached out fraction, which render the solution highly viscous. (Moorthy , 1995) Finally the opacity of the starch suspension is lost and it attains translucency. Some of the major applications of starch depend on its viscosity.

2.8.6 Gelatinization

In uncooked stage starch granules are insoluble in water and presence as discrete particles held together by a network of associated molecules (amylose and amylopectin) by hydrogen bonds. It forms a temporary suspension of starch particles in water and will settle to the bottom of the container unless agitated. When heating a starch suspension with gentle agitation, granules imbibe water and their constituents become hydrated. Therefore starch granules lose their birefringent properties and swell. As heating further granules take up more water irreversibly, the short chains of amylose leached out. Therefore the opacity of granules is lost and increased the viscosity. Ultimately a thick paste is formed. This overall process is known as "gelatinization".

There are several factors affect the swelling characteristics of starch and gelatinization temperature.

- The size and shape of the granule
- Crystalline nature of the starch
- The amylose / amylopectin ratio
- The distribution and degree of branching of the amylopectin
- Amount and type of impurity present

The gelatinization temperature range of some starches is included in the table 2.5

Table 2.5 Gelatinization temperatures of some starches

Starch	Gelatinization temperature range ^0C
Corn	62-74
Wheat	52-64
Rice	61-78
Cassava	52-64
Potato	59-69

Source: (McNicol *et al.* , 1972)

If heating is maintained together with stirring the viscosity soon begins to fall as the integrity of the granules lost. When the paste is allowed to cool, the viscosity rises again and gives a gel like consistency as a result of re-establishing the hydrogen bonding relationships between both amylopectin and amylose. These changes can be observed with the viscometers such as the Barbender Amylograph. (Zapsalis and Beck, 1986)

2.8.7 Hydrolysis of starch

Hydrolysis is an important method that can be used to fragment the starch molecules. Hydrolysis includes acid hydrolysis and enzyme hydrolysis. These methods have industrial importance and nutritional significance, and they offer a more discriminative method of polysaccharide fragmentation.

2.8.7.1 Acid hydrolysis

The primary objective of acid hydrolysis is to randomly fragment starch molecules thereby improving their desired functional properties for specific food application. It is important in industrial carbohydrate processing.

This process can be controlled with in limits to produce significant amounts of D-glucose, disaccharides, oligosaccharides or mixture of these carbohydrate forms.

2.8.7.2 Enzyme hydrolysis

The random hydrolysis of starches by acid is overshadowed by enzymatic hydrolysis. In this method enzyme (hydrolases) are used to attack to glycosidic bonds on starch molecules.

- ∝-amylases :- These are classified as endoglucosidases because they randomly attack amylose at ∝ 1-4 linkages to produce simple sugars such as maltose, glucose and dextrines. ∝-amylses are widely distributed among plants and animals (e.g.:- saliva, pancreatic secretions) and are activated by the presence of chloride ions.

- β-amylases :- These enzymes exhibit exoglycosidic activity and attack the nonreducing end of amylose and produce maltose. It presents in plants like barley malt, sweet potatoes, wheat and soybeans.

- ∝ 1-6 glucosidase :- The ∝ 1-6 glucosidic bonds which are responsible for molecular branching in amylopectin cannot be hydrolyzed by above enzymes. Morever, the use of β-amylases on amylopectin results in hydrolysis of the linear molecular portion into two or three glucose residues of the ∝ 1-6 linkage. These limit dextrins can be enzymatically hydrolyzed at the ∝ 1-6 positions by the action of ∝ 1-6 glucosidase. Therefore enzymes are called debranching enzymes.

- Glucoamylase :- It displays the joint hydrolysis properties of ∝ and β amylases. This enzyme consecutively hydrolyzes ∝ 1-4 linkages found in starches andliberates glucose residue held in ∝ 1-6 linkages at a somewhat lower rate. (Zapsalic and Beck , 1986)

2.8.8 Retrogradation

When the aqueous solutions of amylose leached from starch granules during heating are allowed to stand for a few hours, they begin to show changes in their rheological properties. Cooling of amylose solution leads to formation of solid, sphero-crystalline molecular association that effectively excludes solvent. Ultimately it may leads to formation of a two-phase system ; solid-starch and liquid-solvent.

The aggregation of molecules according to these events termed as retrogradation. (Zapsalic and Beck , 1986)

2.9 Glucose Industry

2.9.1 History

In 1811 Kirchoff discovered that heating starch with dilute sulfuric acid transform it into sugar. The commercial manufacture of glucose sugars from starch began during the Napoleonic Wars with England, when suppliers of sucrose sugar were cut off from France by sea blockade. Then rapid progress of starch sugar production was made in the United States in mid-nineteenth century. (Grace , 1977)

In 1814 Saussure established that starch sugar is identical with grape sugar and correctly explained the mechanism of the process as one of hydrolysis. (Radley , 1976)

2.9.2 Commercial glucose production

Glucose or dextrose is a C_6 sugar found in nature in sweet fruits such as grapes and in honey. The configuration of glucose was discovered by Emil Fisher. (Charles and Brautlecht , 1953)

It is less sweet than sucrose, less soluble in water and cannot be used in every case as a substitute for sugar. At present glucose is usually produced as a syrup or as a solid. Glucose is the common name for the syrups and dextrose for the solids. The physical properties of the syrups vary with the DE and the method of manufacture.

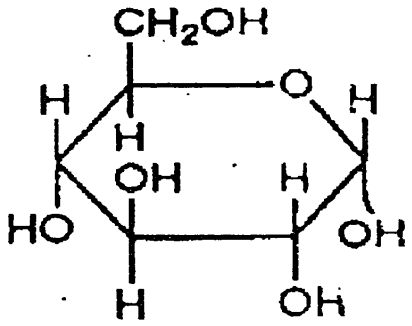


Figure 2.7 Molecular structure of glucose
Source: (Charles and Brautlecht , 1953)

Commercial dextrose is marketed in several forms.

a) Starch syrup / Glucose syrup

It is a thick pale or colourless syrup containing 12-20% moisture and dextrins which prevent crystallization of the dextrose, sucrose or other sugars when used in admixture in confectionary or food products.

b) Crystalline glucose

It appears as a white or pale to brown coloured amorphous mass and is made up of minute crystals. The colour and amount of dextrose are determined by the method of manufacture.

c) Commercial dextrose

It is a fine crystalline powder containing 80-99.5% of pure sugar.

d) Total sugar

It is produced by hydrolysis and marketed as a fine crystalline glucose powder having DE of 96-99. (Jha , 2002)

2.9.3 The Dextrose Equivalent (DE) value

The degree of polysaccharide hydrolysis is expressed in terms of DE. It is the total reducing sugars expressed as glucose and calculated as percentage of the total dry substance. A DE value of 0.0 corresponds to unmodified starch and a DE of 100 represents complete transformation of raw starch to dextrose i.e. D- glucose. Starch hydrolysis products having an approximate DE of ≥ 20 are classified as glucose syrup. The products which has a DE value of < 20 is called maltodextrin. (Zapsalis and Beck , 1986)

According to the DE value, glucose syrups can be classified as follow.

- Low conversion syrups – DE 20 - 37
- Medium conversion – DE 38 - 58
- Regular syrups – DE 43
- High conversion syrups – DE >59 (Bean and Sester , 1992 & Jha , 2002)

All the syrups are wholly carbohydrates and are fully digestible and nutritive. The degree affects the physical properties of the syrups considerably.

As conversion increases, the syrups become sweeter and less viscous, more readily fermented and more hygroscopic. The lower conversion glucoses are more viscous with grater body, and therefore retard crystallization as well as acting as stabilizer towards foams. (Jha , 2002)

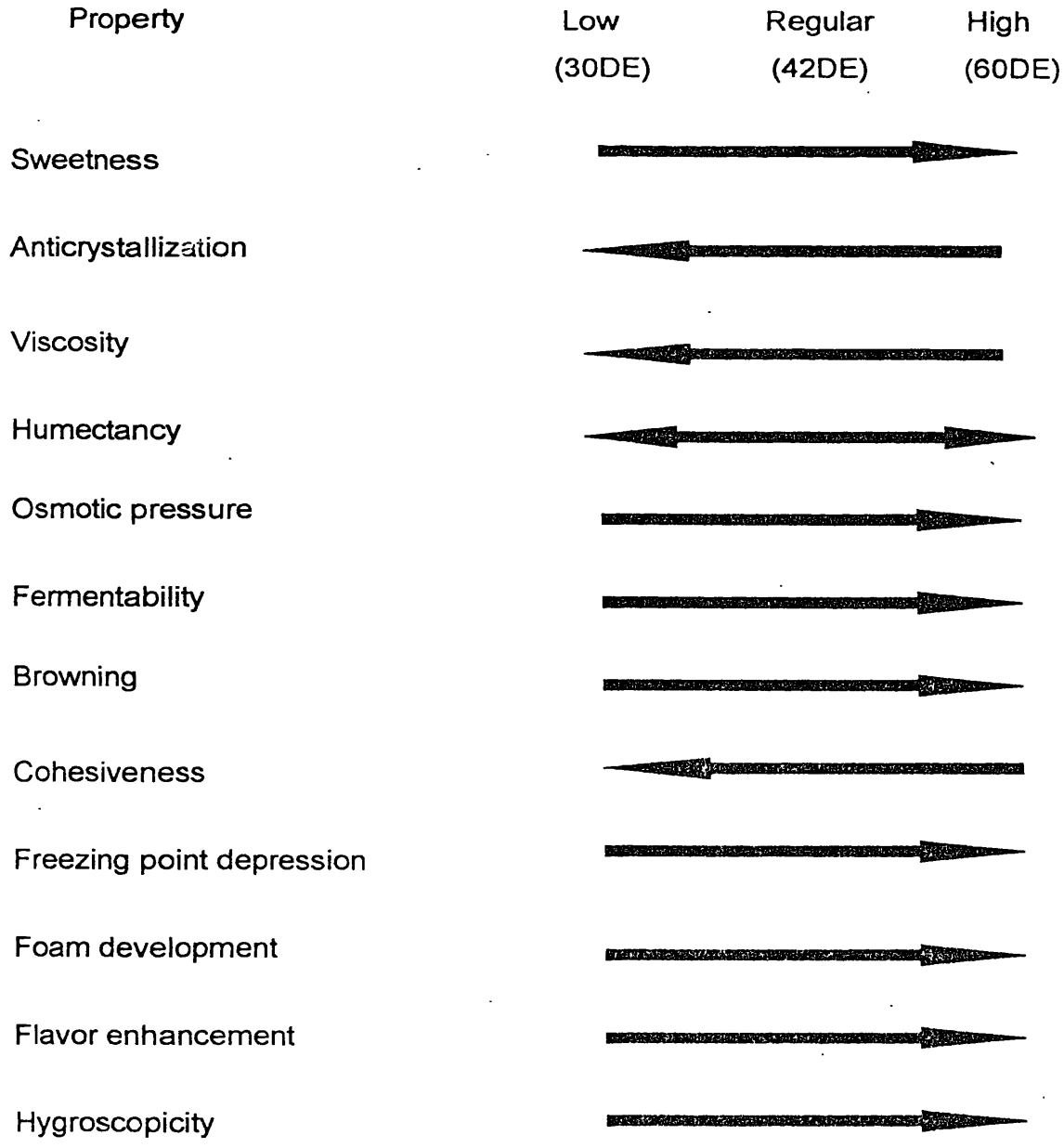


Figure 2.8 Changing of properties of liquid glucose with increasing DE
Source: (Bean and Sester , 1992)

2.9.4 Requirements for liquid glucose

Starch syrup should contains as little as possible otherwise during boiling in confectionery manufacture the sucrose used in conjunction with it will be inverted.

Also sodium carbonate should be used for neutralization when hydrochloric acid has been used since that salt formed doesn't affect the flavour and is inert when used in confectionery. If sulfuric acid is the hydrolytic agent, chalk may be used since the resulting calcium sulphate is readily removed.

For the discoloration of the filtration obtained, can be used charcoal (activated carbon), ion-exchange resins or born char filters. (Radley , 1976)

2.9.5 Uses of liquid glucose

2.9.5.1 Glucose in confectionery

The functional purpose of glucose syrups in boiled sweets, hard candy is to prevent crystallization of the sucrose present and produce a clear non-crystallization product. In addition it contributes to body and mouth appeal. In other types of confectionery other than control crystallization it plays an important part in shelf life and moisture relationships of the sweets when exposed to the atmosphere or stored with other confections of different moisture content.

Fondant making :- Fondant is a basic mix for producing all creamed centers and many other lines in the confectionery field. It consists of a mixture of minute crystals of sucrose, glucose and water. Its consistency is determined by the amount of water left and size and distribution of the sucrose crystals. Traditionally DE 42 glucose is used in fondant making.

Jams, jellies and preserves :- Purposes of adding glucose syrup to jams, jellies and preserves are to control sucrose crystallization, body, appearance, sweetness and osmotic pressure.

2.9.5.2 Glucose in bakery products

Glucose has several functional purposes in yeast raised goods, such as bread and buns. The primary purpose is to supply fermentable carbohydrate on which the yeast may grow and provide CO2 for 'raising' product. Also it gives the flavour derived from residual sweetness and the surface colour derived from the browning reaction to the product.

Use of glucose in baking is greatly expanded as a result of the introduction of the new high fermentable glucose syrups which have a fermentable content of 80% or higher. (Radley , 1976)

2.9.5.3. Glucose in ice cream, ice milk and sherbert

The functional purpose of glucose is to provide a better 'melt down' characteristic, prevent crystallization, improve body and mouth feel and provide balance and sweetness.

There is an increasing trend towards special syrups such as low DE and high maltose because these syrups can be used at much higher levels without any undesirable taste and replace more expensive ingredients such as non-fat milk solids with glucose. (Radley , 1976)

2.9.6 Importation of liquid glucose to Sri Lanka

The amount of liquid glucose imported to Sri Lanka during last years and the annual cost for it are summarized in the following table.

Table 2.6 Annual importation of liquid glucose

Year	Amount (kg)	Cost (Rs.)
1995	7,067,558	73,281,314
1996	3,727,565	83,923,056
1997	3,502,782	84,740,297
1998	3,708,921	88,244,703
1999	1,505,100	33,111,584
2000	1,675,117	100,334,784
2001	3,794,969	114,193,513
2002	4,428,625	139,813,385

Source: (External Trade Statistics , 1995-2002)

Chapter 3

Materials and Methods

3.1 Extraction of starch from raw cassava tubers

Materials :- Cassava tubers
Water

Apparatus :- Stainless steel knife
Cutting board
Trays
Blender (sumeet)
Grater (master CEE)

Method :-

The fresh matured 5 kg of cassava tubers were washed and peeled to remove skin and cortex. The flesh was weighed, washed again and sliced into small pieces using the grater.

Sliced cassava was ground with required amount of water to make cassava pulp. The pulp was put on to a piece of fabric and squeezed by hand to get the starch milk. The extracted starch milk was put on to trays and allowed to settle out. After few hours, the supernatant was decanted and separated the starch. The separated starch was sun dried for about two days. Finally dried cassava starch was scraped, collected, and weighed. Then it was packed and stored in a cool dry place.

The extraction rate of the cassava starch was determined by measuring weight of the cassava starch after drying.

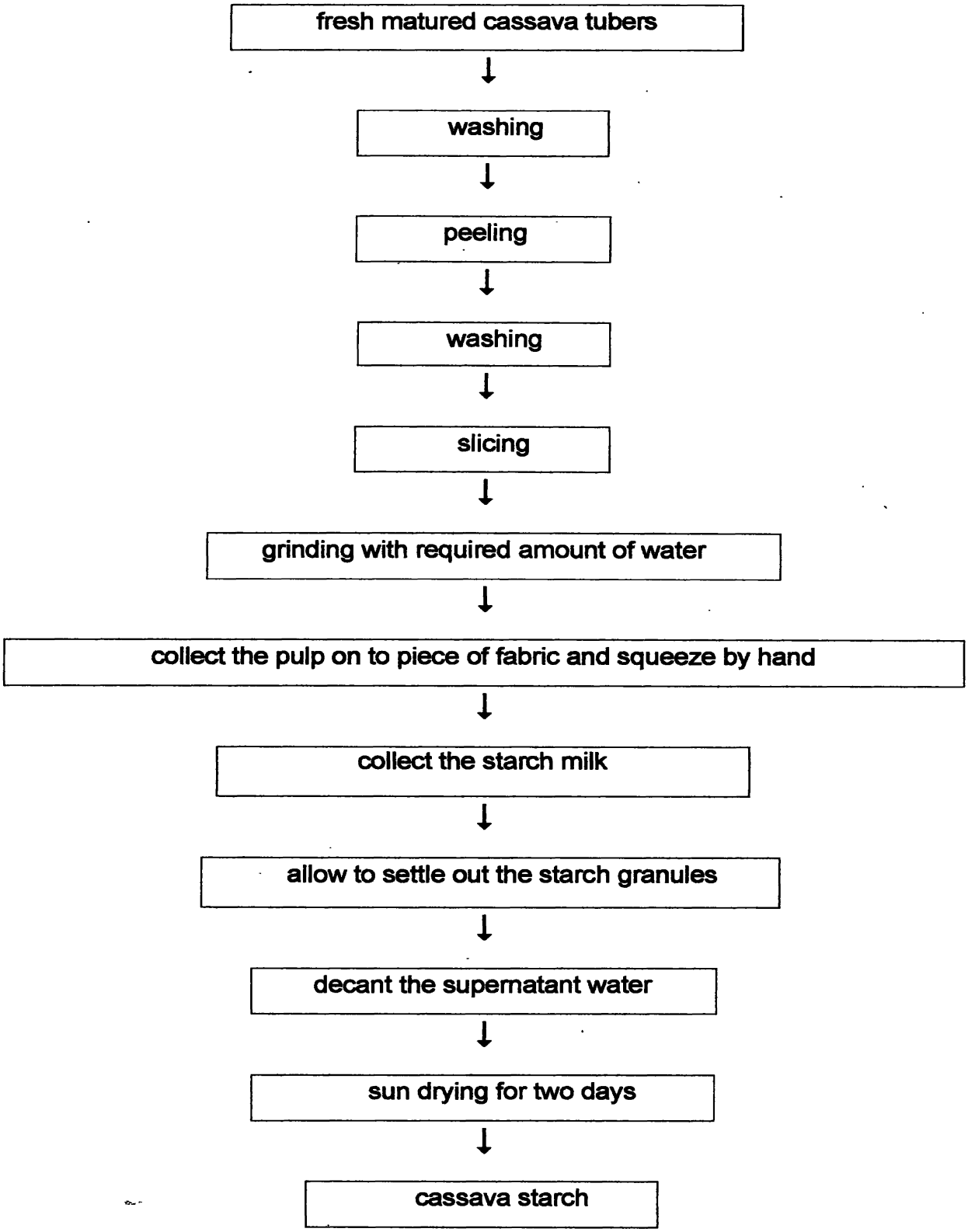


Figure 3.1 Process flow diagram for extraction of cassava starch

3.2 Determination of starch content in cassava tubers

Materials :- Cassava

Apparatus :- Condenser
Other laboratory equipments

Reagents :- 1.125 sp.gr. Hydrochloric acid
5N sodium hydroxide
Fehling's A solution
Fehling's B solution
1% Methylene blue indicator

Sample preparation :-

The peeled cassava tuber was sliced in to small pieces, dried and made into cassava floor. About 2.5 g of cassava flour were mixed with 50 ml water to a smooth suspension and allowed to stand for one hour. It was transferred to a filter and washed with 250 ml of water to remove solubles. The residue was refluxed for 2.5 hours with 200 ml water and 20 ml hydrochloric acid. The content was nearly neutralized with sodium hydroxide and made up to 250 ml. It was filled into the burette. The sugar formed as dextrose was determined by the Lane and Eynon procedure. (Kent – Jones and Amos , 1957)

Method :-

From each Fehling's A and B solutions (solution preparation – appendix B) 50 ml were pipetted out into a beaker and mixed. 25 ml of the above solution was pipetted out into a 250 ml conical flask. 10-15 ml of the sample, which filled into the burette, was added into the conical flask. The content was heated to boil on a heater for two minutes. While boiling the sample was added drop wise from the burette until blue colour change to faint blue. Then 3 - 4 drops of methylene blue indicator was added and completed the titration within 2 - 3 minutes. The burette reading was recorded

and taken the corresponding sugar equivalent from the table given in the appendix A.

Calculation :-

$$\text{Starch content \%} = \frac{X}{100} \times \frac{1}{1000} \times \frac{V}{W} \times 100 \times 0.9$$

X – mg of dextrose per 100 ml

W – weight of the sample

V – volume to which the sample is diluted

3.3 Determination of the presence of HCN in cassava starch

Materials :- Cassava starch

Apparatus :- Platinum wire

Reagents :- Dilute sulfuric acid
Silver nitrate solution
Nitric acid

Method :-

The starch sample was treated with dilute sulfuric acid. A drop of silver nitrate solution containing some nitric acid was put into a loop of platinum wire and held over the evolved gas and observed colour change. (Vogel ,1978)

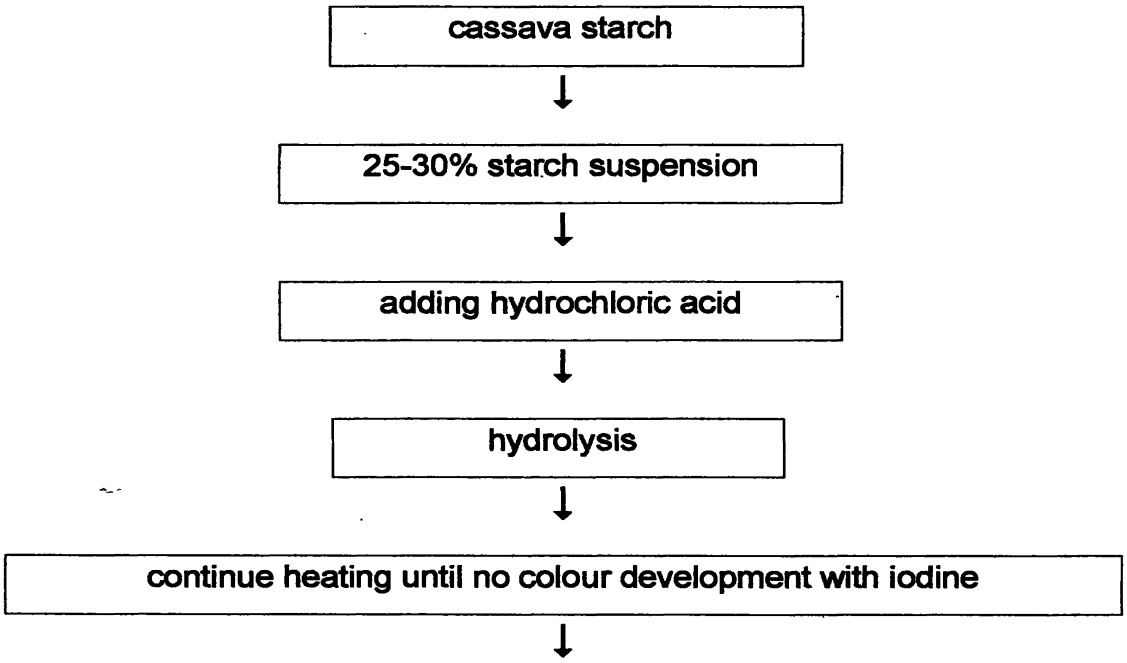
3.4 Preparation of liquid glucose

Materials :- Cassava starch

Apparatus :- Glass bottles
Autoclave (Gallenhome)
pH meter (Cyberscan 2000)
Hand refractometers (range 1-30%, 30-60% and 50-85%)
Bottle sealer
Water bath
Freeze drier (Modulyo)
Other laboratory equipments

Reagents :- Hydrochloric acid
2N sodium carbonate solution
Iodine solution
Activated carbon

Method :-



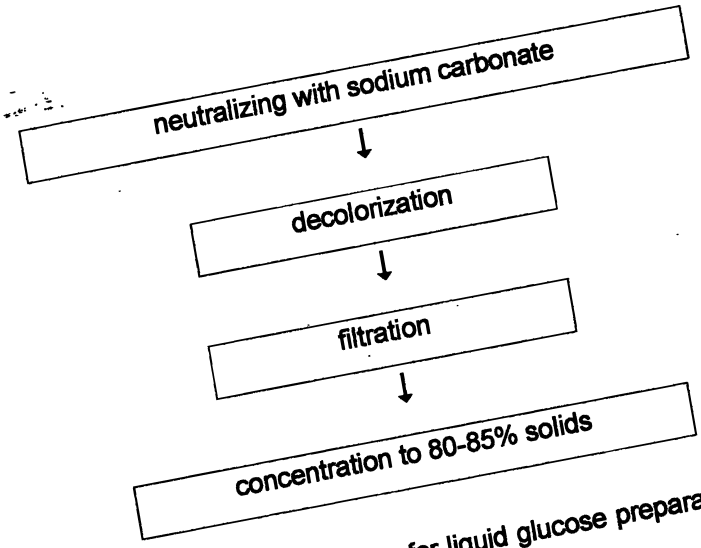


Figure 3.2 Process flow diagram for liquid glucose preparation using cassava starch

Preparation of samples

The starch slurries were prepared by suspending cassava starch in 2- 2 ½ times its weight of water i.e. dispersed 25 g of cassava starch in 100 ml of water for one suspension, and transferred to the glass bottles using a funnel. The suspensions were treated with three different concentrations of hydrochloric acid as described in 3.4.1. Then the bottles were capped and sealed using a bottle sealer. The suspensions were mixed thoroughly.

Hydrolysis

The prepared samples were autoclaved at 120 ^{0}C under three different pressures as given in 3.4.1 to convert starch into glucose. The samples were removed from the autoclave, allowed to cool and added 2 ml of iodine solution which was prepared as given in the appendix B. (Woods and Aurond , 1977) Completion of hydrolysis of the starch suspensions was determined by the no colour development (blue-black) with iodine solution.

Neutralization

The hydrolysates were neutralized with 2N sodium carbonate solution to remove free acid and brought the pH value to 5.0 - 7.0. In this case, pH value of each hydrolysate was measured while adding the sodium carbonate solution to it and adjusted to the required pH.

Decolourization

The neutralized solutions, which were light brown in colour, were decolourized using activated carbon. Sufficient amount of activated carbon (about 10 g) was added to each solution and kept for about 12 hours.

Filtration

The decolourized solutions were filtered through the filter paper held on the funnel to remove solid substances.

Concentration

In the final step the clear filtrate was concentrated until it reached 80-85% solids. Concentration of the samples were done by using two methods i.e. freeze drying and evaporation in the heated water bath.

3.4.1 Determination of the effect of acid concentration and the pressure level on the hydrolysis

The starch suspensions were prepared by dispersing 25 g of cassava starch in 100 ml water. These suspensions were acidified using equal volume (4 ml) of three different acid concentrations, of hydrochloric acid 2M, 4M and 6M respectively. The samples treated with one acid concentration were autoclaved at three different pressure levels 68.94×10^3 Nm^{-2}, 103.41×10^3 Nm^{-2} and 137.88×10^3 Nm^{-2} at 120 ^{0}C. As described, the samples were subjected to nine treatments.

Three replications were done for each treatment and measured the time required for the completion of hydrolysis. The data were statistically analyzed using ANOVA. Also the percentage of solids after hydrolyzing was measured.

3.5 Determination of the physico - chemical properties of liquid glucose

3.5.1 Determination of Total Soluble Solids

The solid percentage of liquid glucose available in the market and prepared liquid glucose using cassava starch was measured using hand refractometer.

3.5.2 Determination the Dextrose Equivalent (DE) value

Sample preparation :-

The 5 g of market liquid glucose sample was dissolved in 50 ml of distilled water and transferred into a 250 ml volumetric flask. Then it was diluted to the mark and mixed thoroughly. 100 ml of the above solution was pipetted out and transferred in to a 250 ml volumetric flask. It was diluted with distilled water and made up to the mark. The sample of prepared liquid glucose was prepared using the same procedure.

Method :-

The DE value of liquid glucose available in the market and prepared liquid glucose sample was measured by Lane and Eynon method as given in 3.2.

Calculation :-

$$\text{Dextrose \%} = \frac{F}{100} \times \frac{1}{1000} \times \frac{V_3}{V_2} \times \frac{V_1}{W} \times 100$$

F – mg of dextrose per 100 ml
V_1 – Volume to which original solution is diluted
V_2 – Aliquot of the solution taken from V_1
V_3 – Volume to which the solution (V_2) is diluted
W – Weight of the sample

3.5.3 Determination of colour

The colour of liquid glucose available in the market and liquid glucose prepared using cassava starch was determined by using colour charts of the Institute of Food Technology (U.S)

3.6 Proximate analysis of liquid glucose

3.6.1 Determination of moisture

Materials :- Prepared liquid glucose sample using cassava starch
Liquid glucose available in the market

Apparatus :- Electronic balance
Oven (Mammert)
Other laboratory equipments

Method :-

The moisture content of both marketed liquid glucose and prepared liquid glucose was determined according to the oven drying method. (Gupta and Varshaney, 1989)

5 g of each sample were weighed into previously weighed petri dishes. The Dishes were placed in an oven at 103 ± 2 ^{0}C for four hours. The dishes were removed from the oven, cooled in a desiccator and weighed. The process of drying, cooling and weighing was repeated at thirty minutes intervals until the difference between the two consecutive weighing was ≤ 1 mg.

Calculation :-

$$\text{\% Moisture content (dry basis)} = \frac{W_2 - W3}{W_2 - W1} \times 100$$

W_1 – mass of the empty dish

W_2 – mass of the sample and dish before drying

W_3 – mass of the sample and dish after drying

3.6.2 Determination of total ash

Materials :- Prepared liquid glucose sample using cassava starch
Liquid glucose available in the market

Apparatus :- Electronic balance
Electric Burner
Muffle Furnace (Gallenbone)
Other laboratory equipments

Method :-

The percentage of total ash was determined according to the following method. (Gupta and Varshaney , 1989)

About 5 g of the samples were weighed into porcelain dishes and heated on a burner until no more fumes evolved. Then the dishes were transferred in to muffle furnace maintained at 500 ± 50 ^{0}C and incinerated for 3 - 4 hours until free from black carbon particles. It was cooled in a desiccator and weighed. The process of incineration, cooling and weighing was repeated at thirty minutes intervals until the deference between two successive weighing was ≤ 1 mg.

Calculation :-

$$\text{Ash content} = \frac{W_2 - W_0}{W_1} \times 100$$

W_0 – mass of the empty dish

W_1 – mass of the sample

W_2 – mass of the sample and dish after ignition

3.7 Comparison of the functional properties of liquid glucose available in the market and prepared liquid glucose using cassava starch

Functional properties of liquid glucose, which is available in the market, and liquid glucose prepared using cassava starch, were compared by preparing jujubes, which is a confectionery product where liquid glucose is used as an ingredient.

Preparation of jujubes

Ingredients :- Sugar 100 g
Gelatin 10 g
Liquid glucose 30 g
Citric acid 0.8 g
Strawberry essence 3 drops
Food colorings (red) 3 drops
Water 40 ml – 20 ml mix with sugar
20 ml dissolve with gelatin

Sugar, gelatin and citric acid were weighed accurately to prepare two jujubes samples. 30 g of liquid glucose from both samples were weighed accurately.

Sugar was added to 20 ml of water and boiled. Market liquid glucose was mixed to the sugar solution when it was boiled. The mixture was boiled up to 127 ^{0}C. Then it was removed from fire. Citric acid, gelatin that dissolved in 20 ml of hot water, colourings and essence were added to the boiled mixture and mixed well. The mixture was poring into oiled mould and left until set. Then the pieces were removed from the mould, cut into small pieces and wrapped with sugar.

Other jujubes sample was prepared using prepared liquid glucose sample using same procedure and the same amount of other ingredients.

Sensory evaluation

A five-point hedonic test was conducted to determine whether a significant difference exists between the two jujubes samples in taste, texture, colour, smell, appearance and acceptability. The samples coded with three digits random numbers in two identical containers were directed to a panel consists with 15 members.

680 – jujubes sample prepared using marketed liquid glucose
504 – jujubes sample prepared using prepared liquid glucose

The samples were simultaneously presented to each panelist with a ballet paper (appendix E) and re-tasting of the samples was allowed. Panelists were instructed to evaluate the each category of the coded samples for degree of liking.

The observed data were analyzed by Man-wittney non-parametric test.

3.8 Cost analysis

The cost for the preparation of 1 kg of liquid glucose using cassava starch was analyzed roughly. The costs for raw materials, power and labour were considered mainly in the cost analysis.

Chapter 4

Results and Discussion

4.1 Extraction of starch

The extracted cassava starch from raw cassava tubers was pure white in colour. As literature states this is due to low content of protein and phenolic compounds in cassava. Starches obtained from other tuber crops are not so white.

Extraction rate of cassava starch = 29.16%

The cassava tubers used in this experiment had occurred vascular streaking. Thus considerable amount of flesh was wasted. Therefore extracted starch content was less than the expected amount.

4.2 Starch content

Among the tuber crops cassava has the highest starch content. Normally cassava contains 25 - 40% of starch. (Moorthy , 1995) The result of the estimation of starch content was observed as 40.5%, which is more closer to the above percentage.

Estimation of starch is done by conversion of starch into sugars by acid hydrolysis. The aldehyde and ketone groups of the sugar have an important property to reduce Fehling's solution while oxidize the sugar into corresponding acid. In the titration cupric oxide in Fehling's solution reduce to cuprous oxide and colour change from blue to brick red. The percentage of dextrose formed can be determined by the Lane and Eynon method and calculate the starch content.

4.3 Presence of HCN

The drop of silver nitrate, held on platinum loop, was not turned into milky colour. HCN was not found in the cassava starch. Formation of silver cyanide, due to the reaction of silver nitrate and HCN results the milky colour.

4.4 Preparation of liquid glucose

For the preparation of liquid glucose, cassava starch has to convert into glucose. Theoretically the conversion of starch to glucose is the summation of the hydrolysis of amylose and amylopectin. However some of the glycosidic linkages in starch appear to be more readily hydrolysed than others. The relative ease of cleavage of the 1-4 and 1-6 glucosidic linkages is still not clear. Generally the 1-4 linkage appears to favor the more rapid hydrolysis but this will have an influence on the speed of the reaction.

The saccharification process begins by gelatinizing the starch slurry and hydrolysis the starch with acid. Both sulfuric acid and hydrochloric acid can be used for this conversion. Temperature and pressure level should be maintained at the required level. Completion of the process is determined using iodine solution. The presence of starch is indicated by developing the blue-black colour when the hydrolysate mixed with iodine solution.

After the completion of hydrolysis the acidified mixture have to be neutralized. When hydrochloric acid use as the hydrolyzing agent, sodium carbonate can be used to remove free acid and obtain a pH value of 5.0 to 7.0. Sodium chloride formed in the syrup in small quantities as a result of the neutralization of the hydrochloric, remains in the solution. However this is not much effect on the confectionery.

When sulfuric acid is used instead of hydrochloric acid as the hydrolyzing agent, calcium hydroxide or sodium carbonate can be used to neutralize it. When use calcium hydroxide, calcium sulfate is formed which is insoluble in water and settle to the bottom. With sodium carbonate, sodium sulfate is formed and remained in the solution.

For the production of satisfactory high quality glucose syrup, the removal of coloured compounds, metallic ions, nitrogenous compounds, organic acids and jel-like particles of unhydrolysed or degraded starch is essential. To remove these

impurities, the liquor can be treated with activated carbon, ion-exchange resins or filtered through the efficient filters such as bom char filters after the neutralization.

As a refining agent activated carbon has very good decolourizing power and striking absorbing power. It absorbs most of the impurities and is a poor absorbent for amino acid. In this experiment activated carbon was used for the purification and the prepared samples gave clear, slightly light yellow colour solutions. Also for the minimum colour formation in the hydrolysis, the temperature should be kept as low and the reaction time as short as possible by using high acid concentration to attain the desired DE.

Glucose syrups heated to high temperatures tend to darken. This darkening is a function of the temperature, glucose concentration and the pH value of the solution. Therefore concentration should be carried out at lower temperature as much as possible. Also Intermediate decolourization during concentration also prevents further darkening.

The resulted samples after the concentration in the water bath were light yellow in colour. This is due to the high temperature involved in the evaporation method. There was no or less colour change observed in the freeze-dried sample. This is due to the low temperature (-60^0C) involved in the freeze drying process. Vacuum evaporation also a best and feasible method, which can be used for effective concentration.

The starch used in the manufacture of glucose syrup must be as pure as possible with low protein content. (Particularly soluble protein) In this respect, cassava starch can be preferable to other starches.

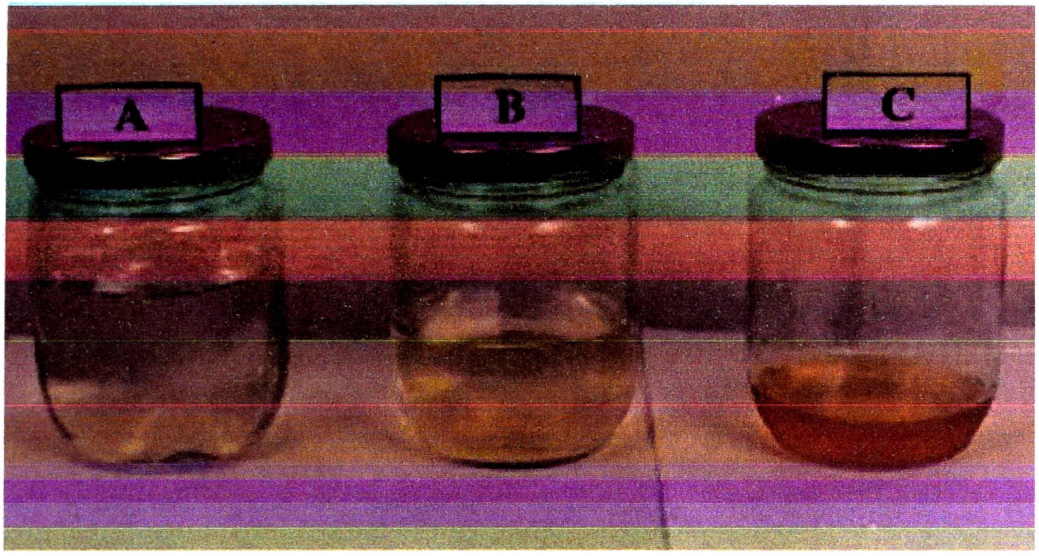


Figure 4.1 Liquid glucose samples

A– Liquid glucose sample available in the market

B–Liquid glucose sample prepared using cassava starch before concentration

C– Liquid glucose sample prepared using cassava starch after concentration

4.4.1 Best acid concentration and pressure level for the hydrolysis

In this experiment some amount of starch samples were subjected to three different acid concentrations and pressure levels. The times required for the completion of the hydrolysis of the samples were given in the table. (Table 4.1)

Table 4.1 Time (in minutes) required for the optimum hydrolysis at different treatments

		Acid concentrations		
		2M	4M	5M
Pressure levels	68.94×10^3 Nm^{-2}	30	20	20
	103.41×10^3 Nm^{-2}	20	15	12
	137.88×10^3 Nm^{-2}	10	7	5

According to the statistical analysis obtained p values for acid concentration and pressure level were 0.007 and 0.000 respectively.

The p value obtained for the acid concentration is less than 0.05 there is an effect of acid concentration on hydrolysis. For the pressure level p value is less than 0.05. There is an effect of acid concentration on hydrolysis.

The best acid concentration and pressure level for the optimum hydrolysis were obtained using mean comparison. (appendix C) 6M acid concentration and $137.88x10^3$ Nm^{-2} pressure level were observed as the best requirements for the optimum hydrolysis.

The Total Soluble Solid (TSS) content of the samples after hydrolysis was given in the table. (Table 4.2)

Table 4.2 TSS content of the treated samples after hydrolysis

Acid concentration	Pressure level	TSS after hydrolysis (%)
2M	$68.94x10^3$ Nm^{-2}	15.4
	$103.41x10^3$ Nm^{-2}	17.5
	$137.88x10^3$ Nm^{-2}	18
4M	$68.94x10^3$ Nm^{-2}	14.6
	$103.41x10^3$ Nm^{-2}	18
	$137.88x10^3$ Nm^{-2}	19
6M	$68.94x10^3$ Nm^{-2}	16.2
	$103.41x10^3$ Nm^{-2}	19.1
	$137.88x10^3$ Nm^{-2}	19.8

4.5 Physico – chemical properties of liquid glucose

4.5.1 Total soluble solids (TSS)

The percentages of solid of liquid glucose available in the market and prepared liquid glucose after concentration were given in the table. (Table 4.3)

Table 4.3 TSS content of liquid glucose

Sample	% TSS
Market liquid glucose	84
Prepared liquid glucose	82

According to the results there is no considerable difference of the percentage solid between the marketed liquid glucose and prepared liquid glucose.

4.5.2 Dextrose Equivalent (DE) value

DE is the measurement of glucose present in the particular sugar syrup and is expressed as the percentage hydrolysis of the glucose bonds. The method used to determine DE is based on the reducing action of the aldose and ketose sugars upon metallic salts such as copper sulfate in fehling's solution.

The DE value was obtained as 37.9 and 39.5 for the prepared liquid glucose sample and liquid glucose available in the market respectively. Those values are less than the standard DE value, which is 43. As the literature states the DE value of the two liquid glucose samples are within the range of medium conversion syrups.

4.5.3 Colour

The liquid glucose available in the market was colourless and the colour of prepared glucose sample was ranged between grayed yellow 162 group C and D.

The observed colour for the prepared liquid glucose sample was darken than the reference sample. This may due to the high temperature involved in the evaporation of the sugar solution

4.6 Proximate analysis of liquid glucose

4.6.1 Moisture content

The results of the quantitative analysis of the moisture content of the liquid glucose available in the market and the liquid glucose prepared using cassava starch are given in the table.

Table 4.4 Moisture content of liquid glucose samples

Sample	Sample weight(g)	Weight of the sample and dish		Moisture content %
		Before drying	After drying	
Market liquid glucose	5.0515	107.9369	107.3223	12.16
Prepared liquid glucose	5.0547	106.2662	105.4310	16.52

Generally glucose syrup contains 12% to 20% moisture. Moisture content of both liquid glucose samples occurred within that range.

The weight loss of the sample due to the evaporation of water is measured in this method. Thus the sample should be spread as a thin layer on a dish to facilitate evaporation. As well as oven temperature must be above the boiling point and should control within 103 ± 2 ^{0}C. Also uniform temperature distribution within the oven will give precise measurements. This is facilitated by the internal fan by circulating the heated air inside the oven.

4.6.2 Total ash

The quantitative analysis of total ash of the liquid glucose available in the market and liquid glucose prepared using cassava starch are given in the following table.

Table 4.5 Total ash content of liquid glucose samples

Sample	Sample weight(g)	Weight of the sample and dish		Ash content %
		Before ignition	After ignition	
Market liquid glucose	5.0973	61.0315	61.0215	0.19
Prepared liquid glucose	5.0624	101.5980	101.4595	2.73

Ash is the inorganic residue remaining after the water and organic matters have been removed by heating at high temperature.

As literature states average composition of ash of liquid glucose is about 0.25 %. According to the results ash content of the prepared liquid glucose sample is higher than 0.25%. The salt formation during the neutralization of the prepared liquid glucose sample with sodium carbonate may be the reason for the high ash content.

4.7 Comparison of the functional properties of liquid glucose available in the market and prepared liquid glucose using cassava starch

The results of the evaluation of the jujubes samples were given in the table.

4.6 Sensory evaluation results for the two jujubes samples

Panelist No.	colour		smell		texture		appearance		taste		Overall acceptability	
	504	680	504	680	504	680	504	680	504	680	504	680
1	5	4	5	4	5	5	5	5	4	5	4	4
2	4	5	3	4	3	4	4	5	3	5	3	4
3	3	4	2	4	3	4	2	4	3	4	3	4
4	5	4	5	5	5	5	5	5	5	4	5	5
5	5	5	4	4	4	5	5	5	5	5	5	4
6	4	5	3	3	5	5	5	5	4	5	5	5
7	3	4	4	5	3	4	4	4	4	4	4	4
8	3	4	4	3	2	3	3	4	2	3	3	4
9	5	4	5	4	5	5	5	4	5	4	5	4
10	5	4	4	4	4	4	4	5	2	4	3	4
11	5	5	5	5	4	5	5	5	5	5	5	5
12	4	4	3	3	4	5	4	4	5	5	5	5
13	5	4	4	5	5	5	5	5	5	3	5	5
14	4	5	3	4	4	5	5	4	5	4	3	4
15	4	5	4	3	4	5	4	3	4	3	4	3

According to the results obtained by the test in appendix D for each category of the two jujubes samples there is no significant difference for colour, smell, texture, appearance, taste and overall acceptability between two jujubes samples. This reveals that there is no significant difference of functional properties between liquid

glucose available in the market and prepared liquid glucose using cassava starch of 5% level of significance.

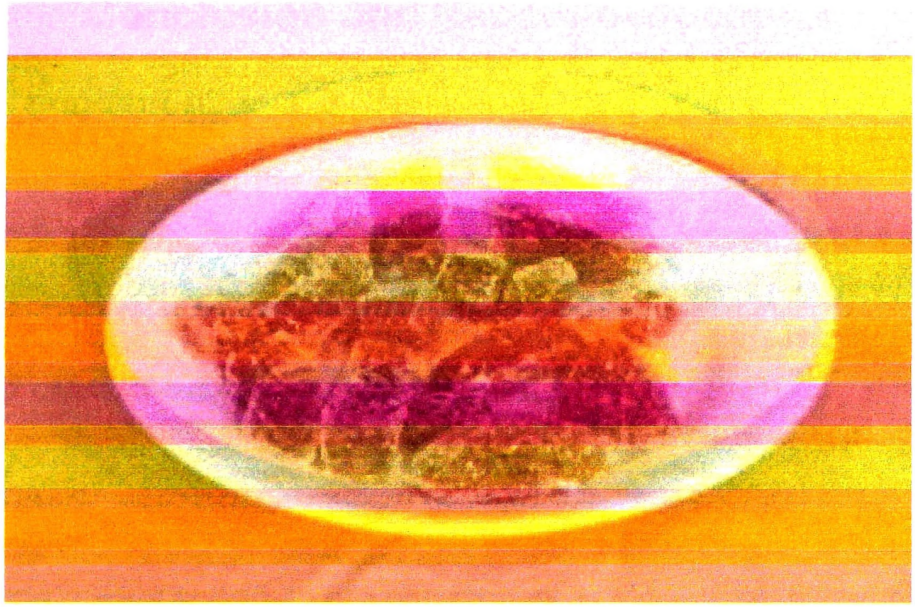


Figure 4.2 The jujubes sample prepared from liquid glucose prepared using cassava starch

4.8 Cost analysis

The cost for the preparation of 1 kg of liquid glucose using cassava starch is as follows.

Raw materials	= Rs. 110.00
Power (Electricity)	= Rs. 30.00
Labour	= Rs. 100.00
Miscellaneous	= Rs. 25.00
	= Rs. 265.00

Chapter 5

Conclusion and Recommendations

5.1 Conclusion

This study was focused on the preparation of liquid glucose using cassava starch. According to the results it can be conclude that liquid glucose can be prepared by acid hydrolysis of cassava starch. Also the statistical analysis reveals that 6M hydrochloric acid and 137.88 x 10^3 Nm^{-2} pressure are the best requirements for the optimum hydrolysis of cassava starch at 120 ^0C.

Statistical analysis of the results obtained from the sensory evaluation reveals that there is no significant difference in functional properties between the liquid glucose available in the market and liquid glucose prepared using cassava starch.

5.2 Recommendations

Analysis of the physico - chemical properties of freeze-dried liquid glucose sample should be carried out.

Studies of shelf life evaluation of the prepared liquid glucose should be done in order to obtain a better quality product.

In this research hydrochloric acid was used as the acidifying agent for the hydrolysis. Therefore studies should be carried out with other acids i.e. sulfuric acid.

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APPENDIX

Appendix A

Lane and Eynon dextrose table

ml of sugar solution required	for 10 ml of Fehling's solution		for 25 ml of Fehling's solution	
	dextrose factor	mg dextrose per 100 ml	dextrose factor	mg. dextrose per 100 ml
15	49.1	327	120.2	801
16	49.2	307	120.2	751
17	49.3	289	120.2	707
18	49.3	274	120.2	668
19	49.4	260	120.3	633
20	49.5	247.4	120.3	601.5
21	49.5	235.8	120.3	572.9
22	49.6	225.5	120.4	547.3
23	49.7	216.1	120.4	523.6
24	49.8	207.4	120.5	501.9
25	49.8	199.3	120.5	482.0
26	49.9	191.8	120.6	463.7
27	49.9	184.9	120.6	446.8
28	50.0	178.5	120.7	431.1
29	50.0	172.5	120.7	416.4
30	50.1	167.0	120.8	402.7
31	50.2	161.8	120.8	389.7
32	50.2	156.9	120.8	377.6
33	50.3	152.4	120.9	366.3
34	50.3	148.0	120.9	355.6
35	50.4	143.9	121.0	345.6
36	50.4	140.0	121.0	336.3
37	50.5	136.4	121.1	327.4
38	50.5	132.9	121.2	318.8
39	50.6	120.6	121.2	310.7
40	50.6	126.5	121.2	303.1
41	50.7	123.6	121.3	295.9
42	50.7	120.8	121.4	289.0
43	50.8	118.1	121.4	282.4
44	50.8	115.5	121.5	276.1
45	50.9	113.0	121.5	270.1
46	50.9	110.6	121.6	264.3
47	51.0	108.4	121.6	258.8
48	51.0	106.2	121.7	253.5
49	51.0	104.1	121.7	248.4
50	51.1	102.2	121.8	243.6

Appendix B

Preparation of iodine solution

Prepare a 2% solution of potassium iodide and add sufficient iodine to colour it a deep yellow

Preparation Fehling's solutions

Fehling's A solution: -From clear crystals of copper sulphate, 34.639 g were measured by using the electric balance. It was placed in a cleaned 500 ml volumetric flask and little amount of distilled water was added. Then it was shaken thoroughly until all the crystals were dissolved. Two drops of concentrated sulfuric were added and then distilled water was added up to the point carefully.

Fehling's B solution: - Sodium Potassium tartarate (Rochelle salt) 173 g and 50 g of sodium hydroxide were measured using electric balance. It was placed in a cleaned 500 ml volumetric flask and about 400 ml of distilled water was added. Then it was shaken thoroughly until it was dissolved. Distilled water was added up to 500ml point carefully. It was allowed to stand overnight and then it was filtered through a muslin cloth.

Appendix C

General Linear Model: responses versus pressure, acid

Factor Type Levels Values
pressure fixed 3 1 2 3
acid fixed 3 1 2 3

Analysis of Variance for response, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
pressure	2	486.89	486.89	243.44	125.20	0.000
acid	2	88.22	88.22	44.11	22.69	0.007
Error	4	7.78	7.78	1.94		
Total	8	582.89				

Tukey 95.0% Simultaneous Confidence Intervals
Response Variable response
All Pair wise Comparisons among Levels of pressure

pressure = 1 subtracted from:

pressure	Lower	Center	Upper	——+——+——+——
2	-13.72	-9.67	-5.61	(——*——)
3	-22.06	-18.00	-13.94	(——*——)
				——+——+——+——
				-18.0 -12.0 -6.0

pressure = 2 subtracted from:

pressure	Lower	Center	Upper	——+——+——+——
3	-12.39	-8.333	-4.276	(——*——)
				——+——+——+——
				-18.0 -12.0 -6.0

Tukey Simultaneous Tests
Response Variable response
All Pair wise Comparisons among Levels of pressure

pressure = 1 subtracted from:

Level pressure	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
2	-9.67	1.139	-8.49	0.0023
3	-18.00	1.139	-15.81	0.0002

pressure = 2 subtracted from:

Level pressure	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
3	-8.333	1.139	-7.319	0.0041

Tukey 95.0% Simultaneous Confidence Intervals
Response Variable response
All Pair wise Comparisons among Levels of acid

acid = 1 subtracted from:

acid	Lower	Center	Upper	----+-------+-------+-------+--
2	-8.06	-4.000	0.058	(-------*-------)
3	-11.72	-7.667	-3.609	(-------*-------)
				----+-------+-------+-------+--
				-10.5 -7.0 -3.5 0.0

acid = 2 subtracted from:

acid	Lower	Center	Upper	----+-------+-------+-------+--
3	-7.724	-3.667	0.3909	(-------*-------)
				----+-------+-------+-------+--
				-10.5 -7.0 -3.5 0.0

Tukey Simultaneous Tests
Response Variable response
All Pair wise Comparisons among Levels of acid

acid = 1 subtracted from:

Level acid	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
2	-4.000	1.139	-3.513	0.0523
3	-7.667	1.139	-6.734	0.0056

acid = 2 subtracted from:

Level acid	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
3	-3.667	1.139	-3.220	0.0680

Appendix D

Mann-Whitney Test and CI: colour 504, colour 680

colour 5 N = 16 Median = 4.5

colour 6 N = 16 Median = 4.0
Point estimate for ETA1-ETA2 is -0.0
95.2 Percent CI for ETA1-ETA2 is (-1.1,1.0)
W = 257.5
Test of ETA1 = ETA2 vs. ETA1 not = ETA2 is significant at 0.8211
The test is significant at 0.8061 (adjusted for ties)

Cannot reject at alpha = 0.05

Mann-Whitney Test and CI: smell 504, smell 680

smell 50 N = 16 Median = 4.0
smell 68 N = 16 Median = 4.0
Point estimate for ETA1-ETA2 is -0.0
95.2 Percent CI for ETA1-ETA2 is (-1.0,1.0)
W = 256.0
Test of ETA1 = ETA2 vs. ETA1 not = ETA2 is significant at 0.7774
The test is significant at 0.7661 (adjusted for ties)

Cannot reject at alpha = 0.05

Mann-Whitney Test and CI: texture 504, texture 680

texture N = 16 Median = 4.0
texture N = 16 Median = 5.0
Point estimate for ETA1-ETA2 is -1.0
95.2 Percent CI for ETA1-ETA2 is (-1.0,0.1)
W = 220.5
Test of ETA1 = ETA2 vs. ETA1 not = ETA2 is significant at 0.1051
The test is significant at 0.0815 (adjusted for ties)

Cannot reject at alpha = 0.05

Mann-Whitney Test and CI: appearance 504, appearance 680

appearan N = 16 Median = 5.0
appearan N = 16 Median = 5.0
Point estimate for ETA1-ETA2 is 0.0
95.2 Percent CI for ETA1-ETA2 is (-1.0,1.0)
W = 259.5
Test of ETA1 = ETA2 vs. ETA1 not = ETA2 is significant at 0.8802

The test is significant at 0.8689 (adjusted for ties)

Cannot reject at alpha = 0.05

Mann-Whitney Test and CI: taste 504, taste680

taste 50 N = 16 Median = 4.5
taste680 N = 16 Median = 4.0
Point estimate for ETA1-ETA2 is -0.0
95.2 Percent CI for ETA1-ETA2 is (-0.9,1.0)
W = 262.0
Test of ETA1 = ETA2 vs. ETA1 not = ETA2 is significant at 0.9549
The test is significant at 0.9525 (adjusted for ties)

Cannot reject at alpha = 0.05

Mann-Whitney Test and CI: acceptability 504, acceptability 680

acceptab N = 16 Median = 4.5
acceptab N = 16 Median = 4.0
Point estimate for ETA1-ETA2 is 0.0
95.2 Percent CI for ETA1-ETA2 is (-1.0,1.1)
W = 257.5
Test of ETA1 = ETA2 vs. ETA1 not = ETA2 is significant at 0.8211
The test is significant at 0.8104 (adjusted for ties)

Cannot reject at alpha = 0.05

Appendix E

Five-point Hedonic scale

Product-Jujubes

Date---------- **Panelist No:----------**

*Assess the sample individually

*Indicate how much you preferred each sample after testing.

*Rinse your mouth with water after tasting each sample.

*Give numerical values ranking from like very much (5) to dislike very much (1) as given below.

Point Scale	Point
Like very much	5
Like slightly	4
Neither like nor Dislike	3
Dislike slightly	2
Dislike very much	1

Sensory Aspects	Sample Code	
	504	680
Colour	.....	.....
Smell	.....	.....
Texture	.....	.....
Appearance	.....	.....
Taste	.....	.....
Overall acceptability	.....	.....

Specific comments:.....

.....

.....

Thank you.

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